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Effect of Preservation Methods of Oil Palm (Elaeis guineensis) Sap and Wine on the Mineral and Vitamin Compositions for Reproductive Health

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Abstracts

Fifteen litres of freshly tapped oil palm sap were collected from a palm wine tapper at Ajuona-Nsukka, Enugu State, in a cooler packed with ice blocks, and divided into three lots. Each lot (5 L) was subjected to either heat treatment (70°C for 40 min to obtain pasteurized palm sap), freezing (- 4°C till frozen), or left untreated to serve as fresh palm sap along with 5 litre of market palm wine, bought from Ogbete Main market, Nsukka. Pasteurized palm sap had significantly ($p<0.05$) higher levels of vitamin A and β-carotene when compared to the other samples probably as a result of concentration due to its lower moisture content. The levels of vitamin A and β-carotene contents decreased in the following order; pasteurized palm sap fresh palm sap, frozen palm sap, and palm wine. The presence of vitamin A in tap water may be as a result of contamination by algae present in the tap hole. Vitamin C content of the samples were 1.48±0.24%, 1.17±0.19%, 0.62±0.00%, and 0.44±0.05 (%) for fresh palm sap, frozen palm sap, market palm wine and pasteurized palm sap, respectively. The mineral contents of the samples showed that copper was not detected in all the samples except in the market palm wine which contained 0.057 $\pm$ 0.001ppm. The presence of copper in the market palm wine may be an indication of contamination of palm wine by the mineral. The equipments for handling of palm sap and wine should therefore be free of copper so as to avoid high concentration of copper in the samples that may be harmful to the health of consuming public. It was asserted that despite the level of palm sap in palm products, the proportional equivalence of any mineral or vitamin in relation to others is more important for its absorption in the blood stream. Also, higher accumulation of any given vitamin and minerals could result to intoxication and thus affect, not only reproductive health, but also overall health indices of an individual.

Keywords: Palm sap, Palm wine, Vitamins, Minerals, Reproduction, Preservation

1. Introduction

Many palms are tapped in the tropics for their saps. Tapping of palm sap from palms such as *Raphia hookeri, Elaeis guineensis, Aranga pinnata, Raphia vinifera,* etc. before fruiting are important since fruiting reduces palm sap production. According to Rangaswami (1977), sugars are intercepted before being used in the production of non-edible parts such as husk in coconut, which represents 35% of the fruit; and in the production of edible materials through chemical reaction, which implies loss, mainly in the conversion of sugar into oil as for coconut and oil palm (Dalibard, 1999). Dalibard (1999) observed that tapping palms for its sap was more economical (profitable) from the point of edible energy production. Although tapping of palms (date palm) was reported in Egypt long before the birth of Christ (Genesis 9: 20 – 21; 14: 18), Dalibard (1999) reported that Hindus knew how to extract palm sap from palms for about 4,000 years ago.

Ikegwu *et al.* (2014) defined palm sap as the unfermented pale-yellow exudate from tapped unopened spathe of oil palm tree (*Elaeis guineensis*) that is consumed as a nourishing beverage in some tropical countries. Tuley (1965) posits that a sgary solution is normally obtained by the excision of the meristem in nearly all palms. As a result of wide yeasts inherent in the palm sap, fermentation commences almost immediately resulting in the production of alcoholic beverage called toddy or palm wine. Palm wine is consumed throughout the tropics. It appears as a whitish liquid produced by natural fermentation of the sap of *Elaeis guineensis* and *Raphia hookeri* (Izuchukwu *et al.* 1991). Palm wine is an alcoholic beverage produced by the spontaneous yeast/lactic fermentation of the sugary sap of palms or may be referred to as a popular traditional alcoholic consumed by more than 10 million people in West Africa (Izuchukwu *et al.,* 2001).

The difficulty of preserving palm sap to prolong the shelf-life has necessitated research on different preservation methods. The problem is attributed to wild inoculum of yeast in palm sap which enables fermentation to take place, converting the sugars to ethanol and subsequently to acetic acid; converting the palm sap to a milky-white product called palm wine that has increased microbial suspension resulting from the prolific growth of the fermentation organism. The action of these yeasts results to loss of sweetness, shortened shelf-life, and decreased acceptability (Odunfa, 1985). Obire (2005) asserted that the fermentation by natural microflora decreases the sugar level rapidly as it is converted to alcohol and other products.

There has been an attempt to preserve palm sap and wine by sulphiting but it failed (Obahiagbon, 2009). Also, health reasons may be a factor to consider in the use of sulphites as it has been associated with cancer. Obahiagbon (2009) believed that pasteurizing palm sap and wine at 70^oC for 40 minutes could preserve the product for 9 months while Obahiagbon and Oviasogie (2007) preserved the sap of *Raphia hookeri* for 24 months without chemical preservative at a pasteurization temperature of 75^oC for 45 minutes. The sap was pasteurized in green bottles and stored under ambient temperature (20 – 25^oC). The present research is a continuation of the works of other researchers and seeks to investigate the effect of different preservation methods on the vitamin and mineral content of palm sap and wine for reproductive health.

2. Materials and methods

Reagents

2.1 Sample procurement and preparation

Fifteen litres of freshly tapped oil palm sap were collected from a palm wine tapper at Ajuona-Nsukka, Enugu State, in a cooler packed with ice blocks, and divided into three lots. Each lot (5 L) was subjected to either heat treatment (70^oC for 40 min to obtain pasteurized palm sap), freezing (- 4^oC till frozen), or left untreated to serve as fresh palm sap along with 5 litre of market palm wine, bought from Ogbete Main market, Nsukka. The frozen palm sap was thawed, and the sample was analyzed along with fresh palm sap, pasteurized palm sap and market palm wine for vitamins and minerals.

2.2 Determination of Vitamin composition of palm sap/wine

Niacin, riboflavin (B_2), β-carotene and vitamin A compositions were determined by the method of AOAC (1990). The method described by AOAC (2010) was used for the determination of vitamins B_1 and C content while Pearson (1976) was used for the determination of vitamin E.

2.3 Determination of Minerals

Samples were ashed according to the methods described by AOAC (2010), and the minerals determined using Atomic Absorption spectrophotometer (AAS) (model number A6800). Five (5) ml of the sample was measured using a syringe into a 250 ml Erlenmeyer flask, acidified with nitric acid and then evaporated to dryness using a steam bath. Fifteen (15) ml of HNO_3 was added and heated in a fume cupboard to a colourless solution after the addition of 5 ml H_2O_2, at 400 – 450^oC for 2 hours. The ashed samples were adjusted to 50 ml volumetric cylinder with distilled water. After ashing, minerals such as cadmium, copper, iron, magnesium, zinc, sodium, potassium, calcium and phosphorous contents were determined according to the method of AOAC (2010).

3. Results and Discussion

The vitamin A content of fresh palm sap was 232.50±10.61 µg/ml and that of pasteurized palm sap was 322.50±3.53. B-carotene contents were 465.00±21.20 µg/ml for fresh palm sap, 435.00±7.06 µg/ml for frozen palm sap, 290.00±0.00 µg/ml for palm wine and 645.00±7.06 µg/ml for pasteurized palm sap. Palm sap and its wine had high contents of vitamin A and β-carotene. Faparusi (1977) reported that high content of vitamin A in palm sap/wine is good for sight. Pasteurized palm sap had significantly ($p<0.05$) higher levels of vitamin A and β-carotene when compared to the other samples probably as a result of concentration due to its lower moisture content. The levels of vitamin A and β-carotene contents decreased in the following order; pasteurized palm sap fresh palm sap, frozen palm sap, and palm wine. The presence of vitamin A in tap water may be as a result of contamination by algae present in the tap hole. A specific requirement for retinol for testicular development, and maintenance of spermatogenesis had been well established (Ahluwalia and Bieri, 1971). According to Ahluwali and Bieri (1971), rats fed retinoic acid grow normally and appeared healthy; however, the testes were reduced to approximately one-half the size in retinol fed rats and spermatogenesis was severely damaged. Coward *et al.* (1966) reported that retinol does not seem to have any direct role in the biosynthesis of androgens. The supplementation of vitamin A with retinoic acid cannot cure infertility and the continued degeneration of retina in male rats, showing that these functions require retinal or retinol, that are intra-convertible, but which cannot be recovered from oxidized retinoic acid. The requirement of retinol to rescue reproduction in vitamin A deficient rats is known to be due to a requirement for local synthesis of retinoic acid from retinol in testis (Moore and Holmes, 1971; Vanpelt and DeRooij, 1991). However, pure vitamin A deficiency may not exist in man, as it may be associated in most cases with protein – calorie malnutrition and infection (O'toole *et al.*, 1974). Therefore, pasteurized palm sap may serve to provide higher levels of vitamin A and β-carotene in diets. The availability of vitamin A in quality and quantity is needed for high motility of the sperm cells.

Vitamin C content of the samples were 1.48±0.24%, 1.17±0.19%, 0.62±0.00%, and 0.44±0.05 (%) for fresh palm sap, frozen palm sap, market palm wine and pasteurized palm sap, respectively. However, there were no significant ($p>0.05$) differences in the levels of vitamin C in fresh palm sap and frozen palm sap, while there was a significant difference ($p<0.05$) between them and market palm wine. Pasteurized palm sap contained the lowest level of vitamin C. The difference was significant ($p<0.05$) when compared to other samples. The difference could be as a result of heat treatment. According to Fellows (2000), vitamin C losses are heat dependent. Vitamin C has been known to serve as antioxidant in diets and its availability in right quantity prevents free radical reactions in the body and improves testicular development.

Table 1: Vitamin content of samples

	Samples			
Vitamins	**Pasteurized palm sap**	**Market palm wine**	**Frozen palm sap**	**Fresh palm sap**
Vitamin A (µg/ml)	322.50±3.53^{a}	145.00±0.00^{d}	217.50±3.53^{c}	232.50±10.61^{b}
Vitamin C (%)	0.44±0.05^{c}	0.62±0.00^{b}	1.17±0.19^{a}	1.48±0.24^{a}
β-carotene (µg/ml)	645.00±7.06^{a}	290.00±0.00^{d}	435.00±7.06^{c}	465.00±21.20^{b}
Vitamin B_1 (mg/ml)	0.26±0.22^{b}	0.15±0.00^{d}	0.21±0.02^{c}	0.32±0.01^{a}
Niacin (µg/ml)	63.00±6.36^{a}	33.00±2.12^{b}	52.50±6.36^{ab}	60.00±16.97^{a}
Vitamin E (mg/ml)	0.59±0.01^{a}	0.20±0.00^{b}	0.25±0.07^{b}	0.20±0.00^{b}

Values are means± S.D. (n = 3). Values with different superscripts within a row were significantly different ($p<0.05$).

Vitamin E and niacin of pasteurized palm sap were significantly ($p<0.05$) higher than those of the other samples. It has been established that vitamin E plays a role as an antioxidant and scavenger of free radicals, making it effective as a protector of the integrity of lipids and phospholipids membrane (Hathcock, 1997). Although pasteurized palm sap had higher levels of vitamin E, its availability beyond recommended levels may pose a health risk and may equally damage organs and tissues. The availability of a given vitamin in food supply must be in proportion to proportional equivalent of other nutrients in the body for it to be effective as a nutraceutical food product and to serve as a fertility enhancer in the body.

Davis and Johnson (1987) detected vitamins C and B_1 in *Borassus flabellifer* and were found to be 13.25mg/100cc and 3.90IU, respectively. Palm wine from sap of *Hyphaene coriacea* was found to contain 6.8mg/100g vitamin C, 0.22mg/100g niacin and 0.01mg/100g riboflavin and 0.01mg/100g thiamin (Cunningham and Wehmeyer, 1988).

The mineral contents of the samples are presented in Table 2. Copper was not detected in all the samples except in the market palm wine which contained 0.057 ± 0.001ppm.

Table 2: Mineral contents of palm sap products

Minerals	**Pasteurized palm sap**	**Palm wine**	**Frozen palm sap**	**Fresh palm sap**
Cu (ppm)	ND	0.057±0.00^{a}	ND	ND
Zn (ppm)	0.075±0.00^{c}	0.085±0.00^{a}	0.078±0.00^{b}	0.064±0.00^{e}
Fe (ppm)	7.01±0.00^{a}	3.02±0.00^{b}	2.06±0.01^{c}	1.34±0.00^{d}
P (ppm)	0.419±0.00^{a}	0.339±0.00^{b}	0.345±0.00^{b}	0.310±0.00^{b}
Mg (ppm)	0.30±0.02^{e}	1.19±0.06^{c}	2.01±0.00^{a}	1.80±0.10^{b}
Ca (ppm)	0.30±0.00^{b}	0.21±0.04^{c}	0.40±0.01^{a}	0.21±0.02^{c}
Cd (ppm)	0.18±0.00^{b}	0.27±0.00^{a}	0.18±0.00^{b}	0.20±0.00^{ab}
K (ppm)	4.45±1.59^{ab}	3.65±0.56^{b}	5.56±2.07^{a}	5.60±3.02^{a}
Na (ppm)	0.49±0.14^{b}	0.39±0.00^{c}	0.59±0.28^{a}	0.49±0.14^{b}

Values are means± S.D (n = 3). Values with different superscripts within a row were significantly different ($p<0.05$). ND – Not detected

The presence of copper in the market palm wine may be an indication of contamination of palm wine by the mineral. According to Gropper *et al*., (2005), copper poisoning could lead to Wilson's disease (copper accumulation in organs causes mutation in gene coding for ATP7B in hepatocytes). Copper functions in the immune system through energy production, neutrology, and activity of antioxidant enzyme production, depth of antioxidant and lymphocytes replication (Niedermann *et al*., 1994; Nockles, 1994). The equipments for handling of palm sap and wine should therefore be free of copper so as to avoid high concentration of copper in the samples that may be harmful to the health of consuming public.

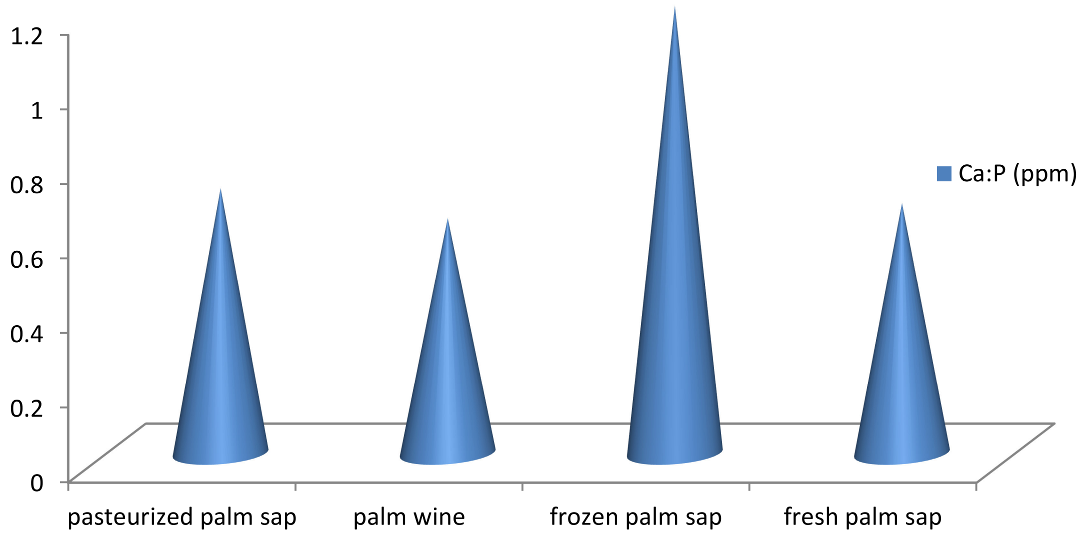

Figure 1 Calcium to phosphorous compositional ratios in the palm sap products

The levels of zinc differed significantly ($p<0.05$) among the samples. Zinc is important in regulating iron uptake and the utilization of iron to make red blood cells (Gropper *et al.*, 2005). Bohoua (2008) reported that the mineral content of palm wine feed (i.e. feed formulated with palm wine), such as calcium, phosphorous and zinc is reinforced by the yeasts, and is responsible for the significant increase in body weight. Market palm wine had the highest content of zinc compared to the other samples. Antagonistic association exists between zinc and cadmium, zinc and iron, and zinc and copper (Gropper *et al.*, 2005). Therefore, the antagonistic relationship between zinc and other mineral components in palm wine, and the possibility that the marketers/tappers used materials made of zinc in handling the product before sale could have been responsible for the high content of zinc in palm wine. The antagonism between copper and zinc deserves special attention because zinc is involved in over 80 different enzyme system functions (Tourma, 1995). Its deficiency can lead to reproductive disorders (Tuorma, 1995). Since most enzymes relating to cell division and replication are zinc-dependent, the time of conception and pregnancy, represents the most vital period for ensuring an optimum zinc status. Fresh palm sap has the least zinc content (0.06 ± 0.00ppm).

The iron content of pasteurized palm sap was found to be significantly ($p<0.05$) higher than those for the other samples. This was followed by the market palm wine, frozen palm sap, control and lastly, fresh palm sap. The differences between the samples were found to be significant ($p<0.05$). Zinc and iron may interact, and negatively affect each other's absorption (Gropper *et al.*, 2005). Likewise, manganese and iron; calcium and iron; calcium phosphate salts and iron; and nickel and iron interact antagonistically in the body. Therefore, determining nutritional interrelationship is much more important than knowing mineral levels alone. In a study carried out by Ikegwu *et al.* (2014) using the same experimental design, high levels of minerals in the sap showed no corresponding increases in the sperm count. However, the number of pups produced by dams mated by male-treated fresh palm sap group showed increased numbers of pups as compared to the number of pups produced by other rat groups. The interrelationship between the minerals in the body played more vital roles in its absorption than their quantity in diets. Ikegwu *et al.* (2014) has shown that the administration of palm sap to young rats or addiction to palm sap with concomitant accumulation of mineral element and alcohol may have contributed to the necrosis observed in the histology of the testes in the treated groups. Though, iron has not been implicated in spermatogenesis, it is needed by pregnant women as a result of their expanding blood volume (Gropper *et al.,* 2005), and the demand of fetus, placenta and blood losses to be incurred in childbirth. Iron also interacts with ascorbic acid in enhancing iron absorption and maintaining iron at the appropriate level. The recommended daily intake for iron is 15 mg/day, and doses larger than 20 mg/day may cause stomach upset, constipation and blackened stools (Beutler and Waales, 2006). Since a high correlation has been established between iron levels in serum and in milk of sows (Spruil *et al.,* 1971), it is evident from the study that palm sap, especially pasteurized palm sap will be needed by lactating mothers, and menstruating women. However, high concentrations of iron have been reported to decrease sperm count (Wise *et al.*, 2003) which agreed with the findings of Ikegwu *et al.* (2014) that pasteurized palm sap administered to male wistar rats showed decreased sperm count as well as the motility of the sperm. Wise *et al.* (2003) reported that a decrease in testicular weight and Leydig cells was as a result of increased serum iron levels. In lactating mothers, pasteurized palm sap would be needed for increased milk flow while menstruating women needs the product for improved menstruation due to the high compositional presence of iron in the sample.

There were significant ($p<0.05$) differences in the levels of phosphorus detected in the samples. Pasteurized palm sap had the highest content of phosphorus followed by the control, frozen palm sap, market palm sap and the fresh palm sap. Phosphorus is second only to calcium in the body (Gropper *et al.*, 2005), and like calcium requires vitamin D for its activity. The proportion of calcium to phosphorus in the bone is 2:1 (Gropper *et al.,* 2005). Results obtained for the different palm sap products showed that the ratios of calcium to phosphorous were 1:1.4, 1:1.6, 1.2:1 and 1:1.5 for pasteurized palm sap, palm wine, frozen palm sap and fresh palm sap, respectively. There has not been any evidence that phosphorus supports reproduction in male animals, but there is strong evidence that consumption of foods high in Cu^{2+} and Zn^{2+} leads to formation of phytates or cation-phytate complexes and prevent copper and zinc respectively from being absorbed (Helferich and Winter, 2000). It is evident from the results obtained that if calcium to phosphorous contents were absorbed in the blood stream in proportion to their composition in the palm sap products, from palm sap will be more effective in the body since its proportion of the mineral was nearly equal to that expected in the bone.

The frozen palm sap contained the highest levels of magnesium and calcium which were significantly ($p<0.05$) higher than the levels found in other samples. According to Silver (2003), calcium levels within the sperm cells increase when it is time for hyperactivation and this spike may be the power surge that helps the sperm to penetrate an egg. When calcium channel was cut off, the sperm lost the power to fertilize the egg. Silver (2003) reported that when the catsper'2 gene in male mice was disrupted, the males became infertile thus giving evidence that in some cases, this gene may contribute to infertility. Magnesium is crucial in the production of healthy sperm and egg and the levels are very high in the semen than in the blood serum. Infertile men have been found to have half the level of magnesium in their semen as fertile men (Sircus, 2007). In a similar study, Ikegwu *et al.* (2014) observed that male wwistar rats had their sperm count and their motility compared favourably with the control ($p<0.05$) which could be attributed to the high content of magnesium composition in the palm sap products administered to them, and may have had effect in mitigating the effect of alcohol in the Leydic cells of the treated rat groups.

The levels of cadmium found in the water, palm wine and fresh palm sap samples were not significantly ($p>0.05$) different from each other, while its content in pasteurized palm sap and frozen palm sap were significantly ($p<0.05$) different from the water and palm wine samples. The observed significant increases ($p<0.05$) in the cadmium composition in pasteurized and frozen palm saps could be due to evaporation of water, and crystallization of water molecules, respectively. This led to increases in the soluble solid components and consequently, increases in cadmium contents in the samples. Chronic administration of heavy metals such as cadmium has been implicated in the reduction of sperm production (Akintoye *et al*., 2006). According to Akintoye *et al.* (2006), a significant ($p<0.05$) negative correlation was observed between serum cadmium levels and all examined biophysical semen characteristics except sperm volume.

Potassium levels detected in the samples showed that there were no significant ($p>0.05$) differences between the frozen sap, and fresh sap and pasteurized palm sap, while significant differences existed among them and the water sample. The market palm wine and the pasteurized palm sap were significantly ($p<0.05$) higher when compared to that of the water sample. Diets high in potassium are associated with lower blood pressure (Gropper *et al.,* 2005).

There were significant differences ($p<0.05$) in the levels of sodium in the samples. Sodium metabolism is regulated by aldosterone. It is the principal cat ion in extracellular fluids. It regulates plasma volume and acid-base balance involved in the maintenance of osmotic pressure of the body fluids, preserves normal irritability of muscles and cell permeability, activates nerve and muscle function and involved in Na^+/K^+-ATPase, maintenance of membrane potentials, transmission of nerve impulses and the absorptive processes of monosaccharide, amino acids, pyrimidine, and bile salts (Soetan *et al.,* 2010). The changes in osmotic pressure are largely dependent on sodium concentration (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al*., 2000). The recommended daily intake of sodium is 2400 mg/day, while a minimum intake of 500 mg sodium/day provides for variations in physical activity and climatic exposure (Gropper *et al.,* 2005). There is paucity of research work to link sodium with reproduction.

4. Conclusion

This study showed that vitamins and minerals had effect on reproductive health. Furthermore, the high abundance of a given vitamin or mineral would have effect in inhibiting the absorption of the other. It is therefore expedient that the complementarity of the nutritional abundance of vitamins and minerals should be studied so as to determine the percentage equivalences of the different components necessary to ensure optimum nutritional performance in the body.

Financial Interest Declaration

The authors wish to state that there are no competing financial interests to declare.

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